Effects of an Anti-Inflammatory Drug on the Brain Structure and Function of Rats with Gulf War Illness

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Introduction

Gulf War Illness [GWI] is a chronic illness that affects approximately 200,000 veterans with fatigue, muscle pain, cognitive problems, insomnia, memory loss, diarrhea, among many symptoms (1). GWI's cause was recently determined to have been subtoxic levels of nerve agents like sarin gas, released into the atmosphere when Iraqi chemical weapon storage facilities were bombed (2). Over time, these airborne nerve agents can inflame the brain and impair its normal function. A key factor behind this process is the cytokine tumor necrosis factor [TNF], which can cause cell death and inflammation if it takes on a soluble form [solTNF]. (3) To solve this problem, we are using an anti-inflammatory drug (known here as drug X) developed to neutralize solTNF, in a rat model of GWI to determine if it should be used in patients with GWI to repair their brain structure and function.

GWI Model and Treatment

Sixty-eight Sprague-Dawley rats in 3 cohorts were used in this study. At 9 weeks of age, half the rats ('GWI' rats) were randomly chosen to be injected with a derivative of sarin called diisopropyl fluorophosphate [DFP] at low levels (0.5 mg/kg, LD_{50}). The other rats ('Naïve' rats) received vehicle injections (ice temperature phosphate buffered saline, or PBS). These injections occurred daily for 5 consecutive days. GWI-similar conditions were allowed to develop in the rats for the next 6 months. This DFP rat model of GWI resembles the current status of veterans with GWI in age, neuronal injury, and behavioral outcomes.

Half the rats in both the GWI and Naïve groups were then injected with the anti-inflammatory 'Drug X' at 10mg/kg. These injections occurred on days 1, 4, 8, and 11, or twice a week for two weeks. The untreated control rats received vehicle injections (PBS). The rats were thus divided into 4 groups, Naïve+Veh, Naïve+Drug, GWI+Veh, and GWI+Drug.

Two weeks later, Open Field (all cohorts) and Novel Object Recognition (cohorts 2&3) Tests were conducted to assess the rats' anxiety and memory. MRI (cohort 3) was performed before the rats were euthanized.

All animal use procedures were approved and in accordance with the VCU Institutional Animal Care and Use Committee and the NIH's Guide for Care and Use of Laboratory Animals.

Behavioral Testing and Methods

Open Field Test (OFT)

The Open Field Test uses a circular open-field arena (diameter 120 cm) with 30 cm high plexiglass walls. Rats were placed into the arena's peripheral zone and their behavior was observed for 5 minutes. The ANY-maze software divided the arena into central (diameter 50 cm) and peripheral zones, and noted the time each rat spent in the central zone.

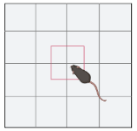

Figure 1: An illustrative diagram of OFT

Novel Object Recognition (NOR) Test

The Novel Object Recognition Test uses the same arena as the OFT. Two identical ceramic garden frog figurines (height ~10 cm) were placed on opposite sides of the arena, 20 cm from the edge. Each rat was placed into the center of the field and were given 5 minutes to explore their surroundings before being returned to their cage. One of the frog figurines was then removed and replaced with a ceramic garden tree figurine (height ~10 cm).

After 1 hour, the rat was again placed into the center of the arena and allowed to explore for 5 minutes. The ANY-maze software tracked the time the rats spent exploring the old object and novel object.

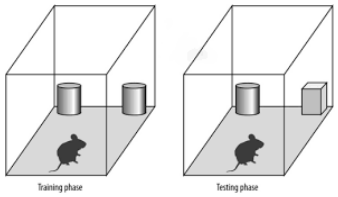

Figure 2: An illustrative diagram of the NOR Test

Magnetic Resonance Imaging (MRI)

The rats in cohort 3 were first deeply anesthetized under 4% isoflurane. MRI was performed on a 7-Tesla 30 cm horizontal bore magnetic resonance imager with a maximum gradient strength of 600 mT/m. Radiofrequency transmission and reception were performed with coils specifically designed for rat brain imaging. DTI scans were acquired using the following parameters: TR/TE of 3000/27 ms, matrix size of 128x128, 20x20 mm field of view, slice thickness of 0.1 mm, and diffusion sensitizing b-values of 0 and 800 s/mm^2. Each DTI scan was assessed through the ImageJ software (National Institute of Health) for brain size, and either hippocampal edema (slice 4) or lateral ventricular size (slice 5).

Results

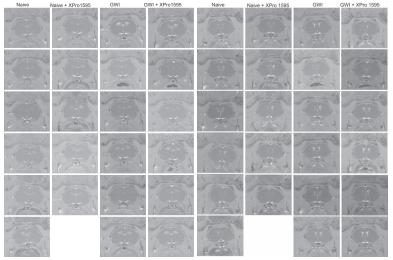

Figure 3: Cohort 3 MRI Scans (Slice 4 on left, Slice 5 on right)

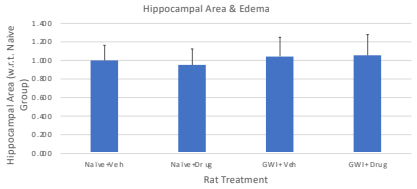

Figure 4: Hippocampal Edemas caused by Treatments (Slice 4 MRIs)

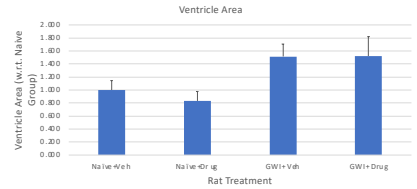

Figure 5: Lateral Ventricle Sizes caused by Treatments (Slice 5 MRIs)

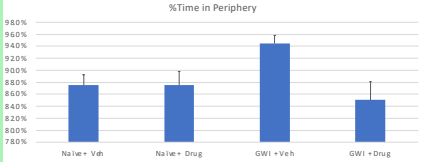

Figure 6: Percent Time Spent in Peripheral Zone in OFT

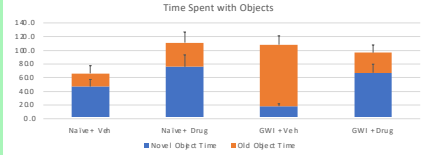

Figure 7: Time Spent with Old and Novel Objects in NOR

Conclusion

Through ImageJ analysis of the MRIs, it was noted that neither exposure to DFP nor Drug X altered the rats' overall brain size. However, exposure to DFP significantly increased the hippocampal area of the GWI+Veh rats through inflammation and development of edema. GWI+Veh rats also had much larger lateral ventricular areas compared to the control (Naïve+Veh). Moreover, two weeks of Drug X treatment for GWI rats had no effect on reducing their irregular hippocampal edema and lateral ventricular size.

Rats should be naturally curious, but behavioral tests found that GWI+Veh rats spent less time in the OFT central zone than control rats, indicating increased levels of anxiety. GWI rats also have difficulty in cognition and memory, because they spent less time exploring the NOR novel object compared to Naïve rats. Treatment with Drug X reversed these impairments (GWI+Drug).

This study demonstrates the efficacy of Drug X in neutralizing TNF and mitigating GWI symptoms in a DFP rat model. To continue developing treatment for veterans with GWI, other anti-inflammatory drugs similar to Drug X could be investigated.

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